

N LEOTIDE SEQUENCES AND PROTEIN QUENCES Nem et al. Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1

FIG. 1A

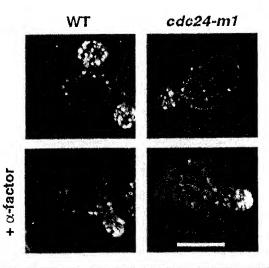


FIG. 1B

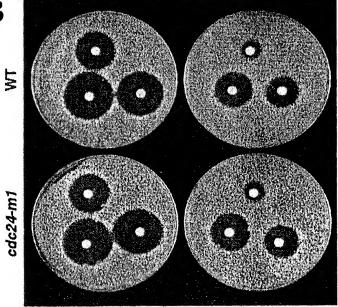
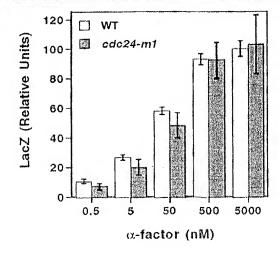


FIG. 1C



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FIG. 2A

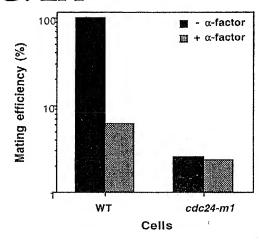
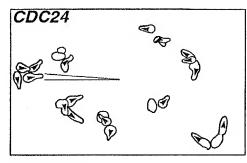
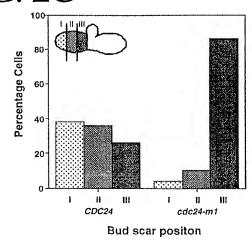


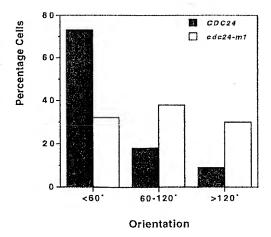
FIG. 2B



Cdc24-m1

FIG. 2C





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FIG. 3A

FIG. 3B

AD STE4 CDC42 BEM1



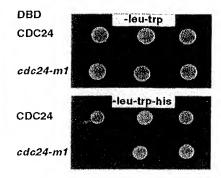


FIG. 3C

Cdc24

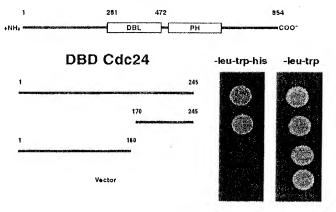
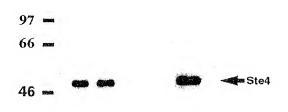
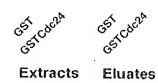


FIG. 3D





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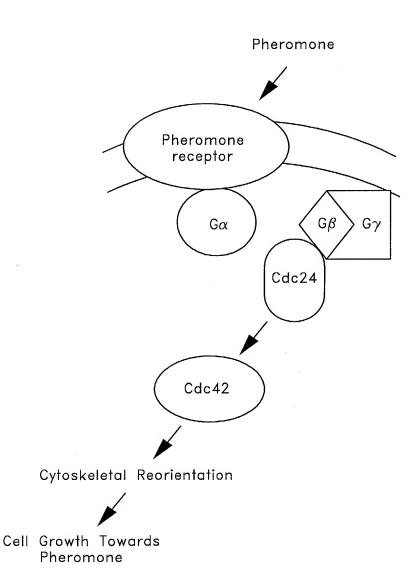


FIG. 4



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FIG.  $5_A$ 

FIG. 5<sub>B</sub>

FIG. 5

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	2113/1			~~1		~~~	ama	202	7.07	2143	711	200	C 2 2	mc x	N COTT	שניא	mam	mma	ייות א
	ATG GAA	H	P	P	A A	A	L	AGA R	T	F	S		O	S	T	S	S	L	N N
	M E 2173/21		P	P	м			K	•	2203		-	¥	_	•	_		_	• •
10	TCA GTA	AGT	ACT	GTT	TCG	TCT	TCA	AGA	ATT	GTT	TCT	CTG	GGC	CCA	GTC	AAT	ATA	AAC	AAT
••	s v	S	T	V	s	S	S	R	I	V	S	S	G	P	V	N	I	N	N
	2233/41									2263	/51								
	TTC AAT	AAA																	
	F N	ĸ	P	s	T	P	K	D	H	L	F	Y	R	C	E	S	L	ĸ	R
15	2293/61 AAA CTA	~		3 mg	O CITT	000	n ma	~~~	003	2323	771	אאכי	ርግ አ	COT	ጥጥር	מאת מ	CAG	CCM	CAA
			AAA K	I	P	G	M	E	P	F	L	N	Q	A	F	N	Q	A	E
	K L 2353/81	Q	K	_	F	G	1-1	12	-	2383		4.	×	••	•	••	~	••	
	CAA CTC	AGT	GAA	CAA	CAA	GCA	TTG	GCT	TTG			GAA	AGA	AGC	TAA	GGA	AAT	GGA	CAT
20	Q L	S	E	Q	Q	A	L	A	L	A	Q	E	R	S	N	G	И	G	H
	2413/10	1.								2443	/11:	L							
	AGT AAT																		
	SN	G	K	R	H	Q	S	L	D	G	A 3/131	M	N	R	L	S	V	G	S
25	2473/12: GAT AGT	አርታጥ ኒ	TCG	ልጥሮ	CAA	GGT	тса	TTG	ACA	CGA	ATG	GCC	ACC	AAT	GCG	TCA	ACG	TCA	TCT
2.5	D S	S	S	I	Q	G	S	L	T	R	М	A	T	N	A	S	T	s	S
	2533/141	1			_	-				2563	/153	L							
	TTA ATC	AGT	GGT	ATG	CCA	AAC										GGG	GTT	TTA	CCA
	L I	S	G	M	P	N	N	N	T	L	F	T	F	т	A	G	V	L	P
30	2593/163 GCT AAT	l room	200	ama	C B III	COT	aam	N CC	CATI	2623	TCC	א א א	TTC	mmc.	CAA	CAA	CCC	GCC	CCC
	A N	I	S	V	D	P	A	T	H		W	K	L	F	Q	Q	G	A	P
	2653/183	L		•	_	_				2683	/191	L				-			
	TTT TGT	GTT	CTT	ATC	AAT	CAT	ATC	CTT		GAT	TCC	CAA							
35	F C	V	L	I	N	H	I	L	Þ	D	S	Q	I	·P	V	V	s	S	D
	2713/201 GAC TTG	L	3 170171	maa	20 70 70	7 7 7	TIC N	CTTA	an v an	2743	7211 ••••••	יליטויא גיטיעי	አ ጥጥ	CCC	GTC	AAG	ልሮአ	C A A	ייייייי
	D L	AGA R	I	C	K	K	S	V	Y Y		F	L	I	A	v	K	T	Q	L
	2773/221	1	_	-				-		2803	/231	L						_	
40	AAT TTT	GAT	GAC	GAG	AAT	ATG	TTC	ACT	ATA	TCC	AAT	GTT			GAC			CAA	
	N F	D	D	E	N	M	F	T	I	-	N	v	F	S	D	N	A	Q	D
	2833/247	L			~~ ~		3 mm		7.7.7	2863	/253	COM	ana	m n C	max.	C A C	COM	n Carr	CNC '
	TTA ATC	AAG K	ATT	ATT	D GAT	V	I	AAT N	K		L L	A	E	Y	S	D	A	S	D
45	L I 2893/261		1.	1	D	•	-		10	2923			-	-	-	_	•-	_	_
	CTG GGT	GGT	GGC	GAT	GAA	GAT	GTA	AAT	ATG	GAT	GTT	CAA	ATT	ACC	GAT	GAA	AGA	TCA	AAA
	S G	G	G	D	E	D	v	N	M		V	Q	I	T	D	E	R	S	K
	2953/281	L 				~~~		C	* ~ *	2983	/291	amm	<b>~~</b> *	a	mma	C 2 2	CTIA	אשכו	mem.
50	GTT TTC		GAA E	ATT	I	GAA E	ACA T	GAA E	AGA R			V	Q	D	L	E	L	M	C
30	V F 3013/301	R	-							3043	/311	L							
	AAA TAC	CGT	CAA	GAT	CTA	TTA	GAA	GCC	GAA	AAT	TTG	TCT	TCA	GAA	CAA	ATT	CAC	TTG	TTA
	K Y	R	Q	$\mathbf{D}$	L	I	E	A	E	N	Ļ	S	S	E	Q	I	H	L	L
	3073/321	L			~~~			~~~	on men	3103	/331	L	mma	ama	2 2 111	000	mm »	(1 N N	mam.
55	TTC CCA				GAG E	ATT	I	D	F		AGA R	R R	F	L	N	G	L	E	C
	F P 3133/341	N	L	N	E	1	_	D	r	~	/35:		r	IJ	74	G			_
	AAC ATC	AAT	GTA	CCT	ATT	AGA	TAT	CAA	AGA	ATT	GGA	TCA	GTA	TTT	TTA	CAT	GCT	TCT	TTG
	N I		V	P	I	R	Y	~	R	I		S	v	F	I	H	A	S	L
60	3193/361	L								3223	/37:	l.							
	GGC CCT																		
		F	N	A	Y	E	P	W	T.	I	ن ا /39		L	T	A	I	D	L	I.
	3253/381 AAC AAA	GVV	CCT	GCT	таа	ጥጥር	AAA	AAA	TCG				ርጥጥ	тар	CCT	GGG	TTT	GAA	CTT
65	N K	E	A	A	N	L	K	ĸ		S	S	L	L	D	P	G	F	E	L
•	3313/401	L								3343	3/41:	ì							
	CAA TCG																		
	Q S		I	L	K	P	I	Q	R	L			Y	P	L	L	L	K	E
70	3373/421 TTA ATC	71 71 71 1	አ C' አ	TCN	CCA	CAA	ጥለጥ	ጥርን	<b>7</b> 7 7 7		3/43: GAC		CAT	aaa	∆.c.c	ጥሮር	ጥሮ፯	ጥርራ	ACA
70	L I						Y		K					G		S	S	S	T
	3433/441	Ĺ								3463	3/45	1.							
	TCA TTC	AAT	GAA	TTA	TTG	GTG	GCT	AAA	ACT	GCA	ATG	AAA	GAA	TTG	GCA	TAA	CAA	GTC	AAT
	<u>s _ F</u> _	<u>N</u>	E	<u> </u>	<u>L</u>	<u>v</u>	<u>A</u>	<u>K</u> _	T .	_A	M	_K	Е	<u>r</u>	<u>A</u>	<u>N</u>	Q	<u>v</u> _	N



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	3493/461								354.	3/47								
	GAG GCG CAA																	
	E A Q 3553/481	R	R	A	E	N	I	E		ப 3/49:	E	K	L	K	E	R	V	G
5	AAT TGG CGT	GGG	ጥጥጥ	AAT	TTG	GAT	GCT	CAA				тта	TTC	CAC	GGA	CAA	GTT	GGG
<i>3</i> ,	N W R	G	F	N	L	D	A	0		E		L	F	Н	G	0	v	G
	3613/501							-		3/51:						-		
	GTT AAA GAT	GCT	GAA	AAT				TAC	GTT	GCT	TAT	CTT	TTT	GAA		ATC	GTA	TTT
• •	V K D	Α	E	И	E	K	E	Y	V	A	Y	L	F	E	ĸ	1	V	F
10	3673/521	~~~	» mm	G 2 M	~ ~ m	3.00	* * *			3/53:		~~~	~~~	770	* * * *	* ~ ~	220	mmm
	TTT TTC ACA	GAA E	ATT	D	D	ACC T	AAA K	AAA K			AAA K		E	AAG K	AAG K	AGC	AAG K	F
	3733/541	E	1	ע	ט	•	10			3/55:		Q	L	10	10			L
	TCG ACA AGA	AAG	AGA	TCA	ACT	TCA	TCA	AAT				TCG	ACT	ACT	AAT	TTG	TTG	GAA
15	S T R	K	R	S	${f r}$	s	S	N	L	-	s	s	T	${f T}$	N	L	L	E
	3793/561									3/57								
	TCA ATA AAC																	
	S I N 3853/581	N	S	R	K	D	N	T		P 3/591	L	E	L	K	G	R	V	Y
20	ATA TCG GAG	ΔΤΤ	TAT	AAC	тта	TCC	GCA	CCA				GGC	TCA	ACT	CTA	ATC	ATC	TCA
20	I S E	I	Y	N	I	s	A	p		T				T	L	I	I	S
	3913/601									3/613								
	TGG TCA GGT																	
25	W S G	R	K	E	S	G	S	F		L 3/63]		Y	R	S	E	E	A	R
23	3973/621 AAC CAA TGG	GAA	AAG	тст	TTA	CGT	GAT	TTG				GAA	ATG	AAT	AAA	CAA	ATT	CAT
	N O W	E	K	c	L	R	D	L	ĸ		N	E	М	N	ĸ	Q	I	Н
	4033/641									3/651								
2.0	AAG AAG TTA																	
30	K K L	R.	D	s	D	s	S	F	N 4123	T 3/671		D	S	A	I	Y	D	Y
	4093/661 ACG GGT ATT	ΔGT	ACG	TCA	CCA	GTC	AAT	CAA	TCA	ነ/ ወ / ነ ውጥ	CAA	CAA	CAA	TAC	TAT	GAT	CAT	CGG
	T G I	S	T	ŝ	P	v	N	Q		T		Q	Q	Y	Y	D	Н	R
	4153/681								4183	3/691	L							
35	GGC TCT CAC								TCA	TCC	ACT							
	G S H 4213/701	S	S	R	H	H	S	s		S 3/711		L	S	M	M	K	N	N
	AGA GTT AAA	TCT	GGT	GAT	TTG	AGT	AGA	ATA				TCA	ACA	ACA	TTA	GAT	TCT	TTC
	R V K	s	G	D	L	S	R	I		s		S	T	T	L	D	S	F.
40	4273/721									3/731								
	AGT AAC AAC				TCA S			ACC T			CCA P	TCT	TTG L	ATG M	TCT	TCA	GAT D	GCC A
	S N N 4333/741	L	N	G	5	F	N	1	-	и 1/751	-	5	בו	141	3	3	D	^
	ACC AAA ACA	ATT	CCA	ACA	TTT	GAC	GTT	GCA				CTT	TAC	AAA	TCG	ACA	GAA	TTG
45	т к т		P	T	F	D	V	A	I	K	L	L	Y	K	S	$\mathbf{T}$	E	L
	4393/761			ama			~~~	2 000	4423	7771		~~	com m	H3/D 3	0.7.0		n mm	a m cr
	TCA GAG CCA			V	N	A A	Q	I	GAG E	Y		D	L	L	O	K	I	I
	S E P 4453/781	11	1	V	14	n.	v	_		791		_	D		~			-
50	TCC CAG ATT	ATC	ACT	TCG	AAC	TTG	GTG	GCA				TAA	ATT	AGT	CGA	TTG	AGA	TAT
	s Q I	I	T	S	N	L	V	A	D	_		N	I	S	R	L	R	Y
	4513/801	C1 70 70	aan	a v a	comm	ama	2 2 00	mma		/811		a n m	G 2 G	maa	aaa	mm n	CTCC	CITITO
	AAA GAC GAC K D D			GAC D	F		N N	L						TGG W		L		L
55	4573/821		J	-	-	•	~1			/831		~	_	"	-	_	•	
	GAT ATG TTA	ACC					TAC	CAA		TCA	AGC	AAT	GAA	AAA	CGA	CTG		
	D M L	T	S	E	D	F	Y	Q	T	S	S	N	E	K	R	S	V	T
	4633/841	di Cam	ጥር እ															
60	GTG TGG GTT V W V S *		1 GH															
60	V W V S																	



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5	Blas	tp line	up o	f S.c. Cdc24p and C.a. Cdc24p	
	S.c.	Cdc24p:	1	MAIQTR-FA	8
10	C.a.	Cdc24p:	1	M R F+ MEHPPAALRTFSTQ	14
10	s.c.	Cdc24p:	9	SGTSLSDLKPKPSATSISIPMQNVMNKPVTEQDSLFHICANIRKRLEVLPQLKPFLQI S +SL+ + S+ +S N+ NKP T +D LF+ C +++++L+ +P ++PFL	. 66
	C.a.	Cdc24p:	15	STSSLNSVSTVSSSRIVSSGPVNINNFNKPSTPKDHLFYRCESLKRKLQKIPGMEPFLNC	74
15		~		AYQSSEVLSERQSLLLSQKQHQELLKSNGANRDSSDLAPTLRSSSISTATSLMSMECA+ +E LSE+Q+L L+Q++ SNG S D A ++ S S S SL M	
		_		AFNQAEQLSEQQALALAQERSNGNGHSNGKRHQSLDGAMNRLSVGSDSSSIQGSLTRMA	
20		_		ISYTNSNPSATPNMEDTLLTFSMGILPITMDCDPVTQLSQLFQQGAPLCILFNSVKPQF; + T+S S PN +TL TF+ G+LP + DP T L +LFQQGAP C+L N + P	-
				NASTSSLISGMPN-NNTLFTFTAGVLPANISVDPATHLWKLFQQGAPFCVLINHILPDS	
25		•		LPVIASDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQLVKVLEVVETLM +PV++SDDL++CKKS+YDF++ K F+DE +FTIS+VF+++ L+K+++V+ L+ IPVVSSDDLRICKKSVYDFLIAVKTQLNFDDENMFTISNVFSDNAQDLIKIIDVINKLL	
23		•		SSPTIFPSKSKTQQIMNAENQHRHOPQQSSKKHNEYVKIIKEFVATERKYVHDLEILDK	
				S + + + +E K+ +E + TERKYV DLE++ K) EYSDASDSGGGDEDVNMDVQITDERSKVFREIIETERKYVQDLELMCK)	
30				RQQLLDSNLITSEELYMLFPNLGDAIDFQRRFLISLEINALVEPSKQRIGALFMH-SKH	
	C.a.	Cdc24p:	303	RQ L+++ ++SE++++LFPNL + IDFQRRFL LE N V QRIG++F+H S RQDLIEAENLSSEQIHLLFPNLNEIIDFQRRFLNGLECNINVPIRYQRIGSVFIHASLGI	362
35	S.c.	Cdc24p:	363	FKLYEPWSIGQNAAIEFLSSTLHKMRVDESQRFIINNKLELQSFLYKPVQRLCRYPLLV	422
	C.a.	Cdc24p:	363	F YEPW+IGQ AI+ ++ ++ S +++ ELQS++ KP+QRLC+YPLL+F FNAYEPWTIGQLTAIDLINKEAANLKKSSSLLDPGFELQSYILKPIQRLCKYPLLLH	419
40	s.c.	Cdc24p:	423	ELLAESSDDNNTKELEAALDISKNIARSINENQRRTENHQVVKKLYGRV EL+ SS + EL A K +A +NE ORR EN + ++KL RV	
40	C.a.	Cdc24p:	420	ELIKTSPEYSKQDPHGSSSSTSFNELLVAKTAMKELANQVNEAQRRAENIEHLEKLKERV	
	s.c.	Cdc24p:	472	VNWKGYRISKFGELLYFDKVFISTTNSSSEPEREFEVYLFEKIIILFSEVVTKKSASSLINW+G+ + GELL+ +V + +E E+E+ YLFEKI+ F+E+ K +	531
45	C.a.	Cdc24p:	480	GNWRGFNLDAQGELLFHGQVGVKDAENEKEYVAYLFEKIVFFFTEIDDTKKSDKQE	535
		•		LKKKSSTSASISASNITDNNGSPHHSYHKRHSNSSSSNNIHLSSSSAAAIIHSSTNSSDN KKST ++SN+ SSS ++ S NS +	
50		_		KKSKFSTRKRSTSSNLSSSTTNLLESINNSRKE	
		~		$ \begin{array}{llllllllllllllllllllllllllllllllllll$	
		_		NTLPLELKGRVYISEIYNISAPNTPGSTLIISWSGRKESGSFTLR	
55		_		FKNEETRDNWSSCLQQLIHDLKNEQFKARHHSSTSTTSSTAKSSSMMSPTTT $+++$ EE R+ W CL+ L + N+Q + S S+ ++ T S+S ++ +T	
		-		YRSEEARNQWEKCLRDLKTNEMNKQIHKKLRDSDSSFNTDDSAIYDYTGISTSPVNQSTQ	
60	_	-		MNTPNHHNSRQTHDSMASFSSSHMKRVSDVLPKRRTTSSSFESEIKS+ +H S + H S ++ S RV + TT SF + +	
		_		QQYYDHRGSHSSRHHSSSSTLSMMKNNRVKSGDLSRISSTSTTLDSFSNNLNGSPNTTNF	
		_		ISENFKNSIPESSILFRISYNNNSNNTSSSEIFTLLVEKVWNFDDLIMAINSKISN +S + +IP + ++ Y +T SE L+V ++DL+ I S+I SN	•
65		_		SLMSSDATKTIPTFDVAIKLLYKSTELSEPLIVNAQIEYNDLLQKIISQIITSN	787
		-		THNNNISPITKIKYQDEDGDFVVLGSDEDWNVAKEMLAENNEKFLNIRLY 854 ++++ 1++++Y+D++GDFV L SD+DW + +ML + F +  LADDEN 15BL BYNDECDEVAL NEDDWG LV DW TGED - BYDTGGVEKPGVTVHVG	9.4.4
	C.a.	Cdc24p:	788	LVADDVN-ISRLRYKDDEGDFVNLNSDDDWGLVLDMLTSEDFYQTSSNEKRSVTVWVS	844

FIG. 6

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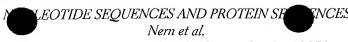
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FIG. 7A<sub>1</sub>

FIG. 7A<sub>2</sub>

FIG. 7A

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FIG. 7A<sub>1</sub>

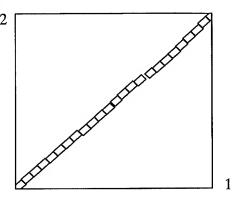
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#### BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.0.9 [May-07-1999]

Matrix 0	BLC	SUM62	<b>+</b>	gap ope	en: 1	1 gap	p exte	ension: $\boxed{1}$	
x_dropoff:	50	expect: 10.	0 w	ordsize:	3	<u>Filter</u>		Align	

Sequence 1 lcl|S.c. Cdc24p Length 854 (1..854)

Sequence 2 lcl|C.a. Cdc24p Length 844 (1 .. 844)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 446 bits (1136), Expect = e-124 Identities = 288/881 (32%), Positives = 464/881 (51%), Gaps = 112/881 (12%)

```
SGTSLSDLKPKPSATSISIPMQNV--MNKPVTEQDSLFHICANIRKRLEVLPQLKPFLQL 66
Query: 9
          S +SL+ + S+ +S N+
                                   NKP T +D LF+ C ++++L+ +P ++PFL
Sbjct: 15 STSSLNSVSTVSSSRIVSSGPVNINNFNKPSTPKDHLFYRCESLKRKLQKIPGMEPFLNQ 74
Query: 67 AYQSSEVLSERQSLLLSQKQHQELLKSNGANRDSSDLAP---TLRSSSISTATSLMSMEG 123
                                                   ++ S S S
          A+ +E LSE+Q+L L+Q++
                                   SNG
                                          SDA
                                                             SL M
Sbjct: 75 AFNQAEQLSEQQALALAQERSNGNGHSNGKRHQSLDGAMNRLSVGSDSSSIQGSLTRMAT 134
Query: 124 ISYTNSNPSATPNMEDTLLTFSMGILPITMDCDPVTQLSQLFQQGAPLCILFNSVKPQFK 183
           + T+S S PN +TL TF+ G+LP + DP T L +LFQQGAP C+L N + P
Sbjct: 135 NASTSSLISGMPN-NNTLFTFTAGVLPANISVDPATHLWKLFQQGAPFCVLINHILPDSQ 193
Query: 184 LPVIASDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQLVKVLEVVETLMN 243
           +PV++SDDL++CKKS+YDF++ K F+DE +FTIS+VF+++ L+K+++V+ L+
Sbjct: 194 IPVVSSDDLRICKKSVYDFLIAVKTQLNFDDENMFTISNVFSDNAQDLIKIIDVINKLLA 253
Query: 244 SSPTIFPSKSKTQQIMNAENQHRHQPQQSSKKHNEYVKIIKEFVATERKYVHDLEILDKY 303
                                       + +E K+ +E + TERKYV DLE++ KY
                      + +
Sbjct: 254 EYSDASDSGGGDEDV------NMDVQITDERSKVFREIIETERKYVQDLELMCKY 302
Query: 304 RQQLLDSNLITSEELYMLFPNLGDAIDFQRRFLISLEINALVEPSKQRIGALFMH-SKHF 362
          RQ L+++ ++SE+++LFPNL + IDFQRRFL LE N V
                                                       QRIG++F+H S
Sbjct: 303 RQDLIEAENLSSEQIHLLFPNLNEIIDFQRRFLNGLECNINVPIRYQRIGSVFIHASLGP 362
Query: 363 FKLYEPWSIGQNAAIEFLSSTLHKMRVDESQRFIINNKLELQSFLYKPVQRLCRYPLLVK 422
          F YEPW+IGQ AI+ ++ ++ S +++ ELQS++ KP+QRLC+YPLL+K
Sbjct: 363 FNAYEPWTIGQLTAIDLINKEAANLKKSSS---LLDPGFELQSYILKPIQRLCKYPLLLK 419
```



#### NEOTIDE SEQUENCES AND PROTEIN SI Nern et al.

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```
Query: 551 --NGSPHHSYHKRHSNSSSSNNIHLS-----SSSAAAIIHSSTNSSDNNSNNSSSS 599
            GPH
                    + S+ + +S
                                             + I S+ ++ N N SSS
Sbjct: 477 FWRGDPQHESFILKLRNEESHKLWMSVLNRLLWKNEHGSPKDIRSAASTPANPVYNRSSS 536
Ouery: 600 SLFKLSANEPKLD-LRGRIMIMNLN---QIIPQNNRSLNITWESIKEQGNFLLKFKNEET 655
            K N D LR + N+N I +++S T + K+
Sbjct: 537 QTSK-GYNSSDYDLLRTHSLDENVNSPTSISSPSSKSSPFTKTTSKDT-----KSATT 588
Query: 656 RDNWSSCLQQLIHDLKNEQFKARHHSSTST----TSSTAKSSSMMSPTTTMNT--PNHH 708
                 +L +
                           r +TST
                                          +SSTA
                                                 `S +S + +N+
Sbjct: 589 TDERPSDFIRLNSEESVGTSSLRTSQTTSTIVSNDSSSTASIPSQISRISQVNSLLNDYN 648
Query: 709 NSRQTH------DSMASF---SSSHMKRVSD-----VLPKRRTTSSSFESE 745
                          S++ F SSS +++ D
                                                    + P++ + S+ +S+
Sbjct: 649 YNRQSHITRVYSGTDDGSSVSIFEDTSSSTKQKIFDQPTTNDCDVMRPRQYSYSAGMKSD 708
Query: 746 IKSISENFKNSIPESSILFRISYNNNSNNTSSSEI----FTLLVEKVWNFDDLIMAINSK 801
                  S+ SS +S N +N + L+V FD+L+ + K
Sbjct: 709 GSLLPSTKHTSLSSSSTSTSLSVRNTTNVKIRLRLHEVSLVLVVAHDITFDELLAKVEHK 768
Query: 802 IS--NTHNNNISPITKIKYQDEDGDFVVLGSDEDWNVAKE 839
              + ++KY DEDGDF+ + SDED +A E
Sbjct: 769 IKLCGILKQAVPFRVRLKYVDEDGDFITITSDEDVLMAFE 808
CPU time: 0.26 user secs. 0.04 sys. secs 0.30 total secs.
Gapped
Lambda
          0.0470
  0.270
Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 10384
Number of Sequences: 0
Number of extensions: 671
Number of successful extensions: 13
Number of sequences better than 10.0: 1
Number of HSP's better than 10.0 without gapping: 1
Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 2
length of query: 834
length of database: 90,077,593
effective HSP length: 61
effective length of query: 773
effective length of database: 83489227
effective search space: 64537172471
effective search space used: 64537172471
T: 9
A: 40
X1: 16 ( 7.3 bits)
x2: 128 (49.9 bits)
X3: 128 (49.9 bits)
S1: 41 (21.7 bits)
S2: 73 (32.8 bits)
```



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FIG. 7B<sub>1</sub>

FIG. 7B<sub>2</sub>

FIG. 7B



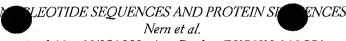


FIG. 7B<sub>1</sub>

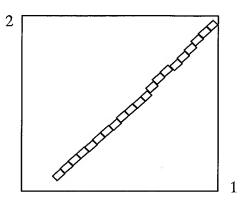
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#### BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.0.9 [May-07-1999]

Matrix 0	BLOSUM62	ap open: 1	gap extension: 1
x_dropoff:	50 expect: 10	0 wordsize: 3	Filter Align

Sequence 1 lcl|S.c. Cdc24p Length 854 (1..854)

Sequence 2 lcl|S.p. Cdc24p Length 834 (1..834)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 238 bits (601), Expect = 1e-61 Identities = 212/760 (27%), Positives = 348/760 (44%), Gaps = 94/760 (12%)

```
Query: 156 DPVTQLSQLFQQGAPLCILFNSVKPQFKLFVIASDDLK---VCKKSIYDFILGCKKHFAF 212
          DPVT++ + G PLC LFN + + KL V +S L+ VCK S+Y F+L CK
Sbjct: 67 DPVTEIWLFCRLGYPLCALFNCLPVKQKLEVNSSVSLENTNVCKASLYRFMLMCKNELGL 126
Query: 213 NDEELFTISDVFANSTSQLVKVLEVVETLMNSSPTIFPSKSKTQQIMNAENQHRHQPQQS 272
           D LF+IS+++ ST+ LV+ L+ +E L+
                                             +KS +
                                                      + ++
Sbjct: 127 TDAALFSISEIYKPSTAPLVRALQTIELLLKKYEVSNTTKSSSTPSPSTDDNVPTGTLNS 186
Query: 273 SKKHNEYVKIIKEFVATERKYVHDLEILDKYRQQLLDSNLITSEELYMLFPNLGDAIDFQ 332
                  ++ E TE KY+ DLE L Y L +++ + + F NL + +DFQ
Sbjct: 187 LIASGR--RVTAELYETELKYIQDLEYLSNYMVILQQKQILSQDTILSIFTNLNEILDFQ 244
Query: 333 RRFLISLEINALVEPSKQRIGALFMHSKHFFKLYEPWSIG-QNAAIEFLSSTLHKMRVDE 391
          RRFL+ LE+N + + + QR+GALF+ + F + Y+ + NA + +
Sbjct: 245 RRFLVGLEMNLSLPVEEQRLGALFIALEEGFSVYQVFCTNFPNAQQLIIDNQNQLLKVAN 304
Query: 392 SQRFIINNKLELQSFLYKPVQRLCRYPLLVKELL-AESSDDNNTKELEAALDISKNIARS 450
                   EL + L KP+QR+C+YPLL+ +LL S
                                                    +EL+ +
Sbjct: 305 ----LLEPSYELPALLIKPIQRICKYPLLLNQLLKGTPSGYQYEEELKQGMACVVRVANQ 360
Query: 451 INENQRRTENHQVVKKLYGRVVNWKGYRISKFGELLYFDKVFISTTNSSSEPEREFEVYL 510
          +NE +R EN + +L RV++WKGY + FG+LL +D V +
                                                        ++ ERE+ VYL
Sbjct: 361 VNETRRIHENRNAIIELEQRVIDWKGYSLQYFGQLLVWDVVNV----CKADIEREYHVYL 416
Query: 511 FEKIIILFSEVVT-KKSASSLILKKKSSTSAS----ISASNITDN----- 550
                  E+ T K+ A S+ + KK+ S
          FEKI++
                                              I SNIT
Sbjct: 417 FEKILLCCKEMSTLKRQARSISMNKKTKRLDSLQLKGRILTSNITTVVPNHHMGSYAIQI 476
```

\$2: 73 (32.8 bits)

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```
Query: 423 ELLAE-----SSDDNNTKELEAALDISKNIARSINENQRRTENHQVVKKLYGRV 471
              SS + EL A K +A +NE QRR EN + ++KL RV
          EL+
Sbjct: 420 ELIKTSPEYSKQDPHGSSSSTSPNELLVAKTAMKELANQVNEAQRRAENIEHLEKLKERV 479
Query: 472 VNWKGYRISKFGELLYFDKVFISTTNSSSEPEREFEVYLFEKIIILFSEVVTKKSASSLI 531
           NW+G+ + GELL+ +V + +E E+E+ YLFEKI+ F+E+ K +
Sbjct: 480 GNWRGFNLDAQGELLFHGQVGV----KDAENEKEYVAYLFEKIVFFFTEIDDTKKSDKQE 535
Query: 532 LKKKSSTSASISASNITDNNGSPHHSYHKRHSNSSSSNNIHLSSSSAAAIIHSSTNSSDN 591
           K K ST ++SN+
                                                   SSS ++ S NS +
Sbjct: 536 KKSKFSTRKRSTSSNL-----
                                               ----SSSTTNLLESINNSRKD 568
Query: 592 NSNNSSSSSLFKLSANEPKLDLRGRIMIMNLNQIIPQN--NRSLNITWESIKEQGNFLLK 649
                            L+L+GR+ I + I N
                                                  +L I+W
                                                         KE G+F L+
                 -----LPLELKGRVYISETYNISAPNTPGSTLIISWSGRKESGSFTLR 613
Sbjct: 569 NT--
Query: 650 FKNEETRDNWSSCLQQLIHDLKNEQFKARHHSSTSTTSS-----TAKSSSMMSPTTT 701
          +++EE R+ W CL+ L + N+Q + S S+ ++ T S+S ++ +T
Sbjct: 614 YRSEEARNQWEKCLRDLKTNEMNKQIHKKLRDSDSSFNTDDSAIYDYTGISTSPVNQSTQ 673
Query: 702 MNTPNHHNSRQT--HDSMASFSSSHMKRVS----DVLPKRRTTSSSFESEIKS----- 748
              +H S + H S ++ S RV + TT SF + +
Sbjct: 674 QQYYDHRGSHSSRHHSSSSTLSMMKNNRVKSGDLSRISSTSTTLDSFSNNLNGSPNTTNP 733
Query: 749 -- ISENFKNSIPESSILFRISYNNNSNNTSSSEIFTLLVEKVWNFDDLIMAINSKI--SN 804
            +S + +IP + ++ Y +T SE L+V ++DL+ I S+I SN
Sbjct: 734 SLMSSDATKTIPTFDVAIKLLY----KSTELSE--PLIVNAQIEYNDLLQKIISQIITSN 787
Query: 805 THNNNISPITKIKYQDEDGDFVVLGSDEDWNVAKEMLAENN 845
             ++++ I++++Y+D++GDFV L SD+DW + +ML
Sbjct: 788 LVADDVN-ISRLRYKDDEGDFVNLNSDDDWGLVLDMLTSED 827
                                0.02 sys. secs
                                                        0.28 total secs.
           0.26 user secs.
CPU time:
Gapped
Lambda
          K
          0.0470
   0.270
                    0.230
Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 12253
Number of Sequences: 0
Number of extensions: 709
Number of successful extensions: 15
Number of sequences better than 10.0: 1
Number of HSP's better than 10.0 without gapping: 1
Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 1
length of query: 844
length of database: 90,077,593
effective HSP length: 63
effective length of query: 781
effective length of database: 83353792
effective search space: 65099311552
effective search space used: 65099311552
T: 9
A: 40
X1: 16 ( 7.2 bits)
X2: 128 (49.9 bits)
X3: 128 (49.9 bits)
S1: 42 (21.9 bits)
```



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Sc KLPVIASDDLKVCKKSIYDFIL (SEQ ID No 25) ++PV++SDDL++CKKS+YDF++ Ca QIPVVSSDDLRICKKSVYDFLI (SEQ ID No 26)

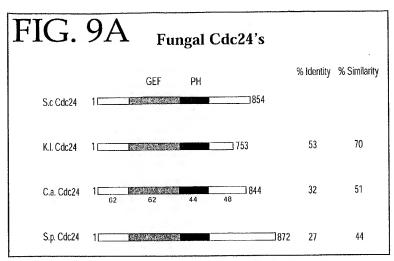
 $Sc = Saccharomyces\ cerevisiae$ 

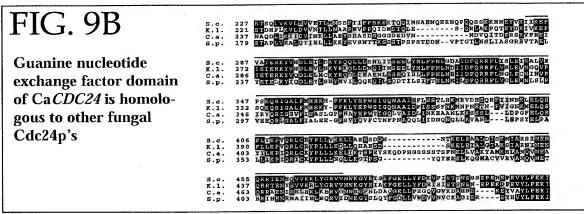
Ca = Candida albicans

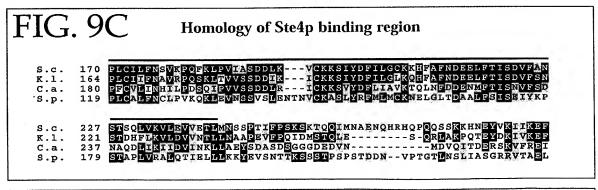
FIG. 8



#### N LEOTIDE SEQUENCES AND PROTEIN NUENCES Nem et al. Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1







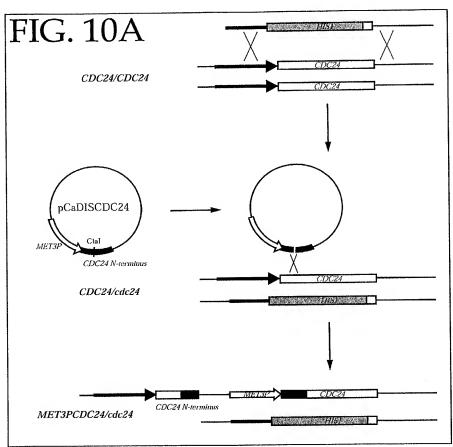
```
Homology of Bem1p binding region

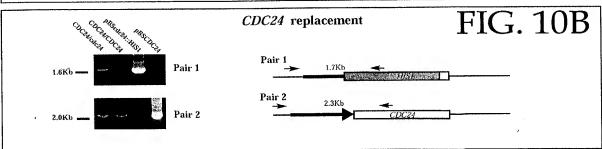
S.C. 774
K.1. 789
C.a. 746
S.P. 768
SVRNTTNVKIRLRLHEVSLVEVVVAHDITFDELLAKVEHKIKLGILKQAVPFRVRLKYVD

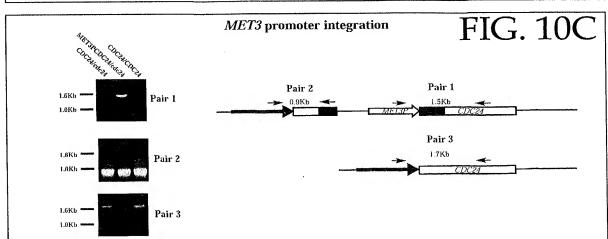
S.C. 822
K.1. 723
C.a. 804
S.P. 828
EDGDFVM-SDDDWGLVLDMLTSEDFYQTSSNEKRSVTVWVS---
EDGDFITITSDEDVLMAFETCTFELMDPVHNKGMDTVSLHVVVYF
```

08/30/02

N EOTIDE SEQUENCES AND PROTEIN VUENCES
Nem et al.
Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1





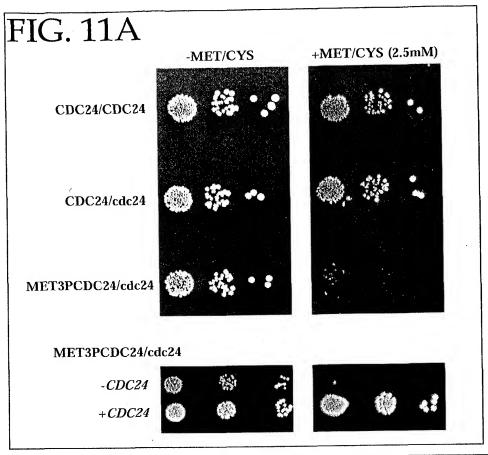


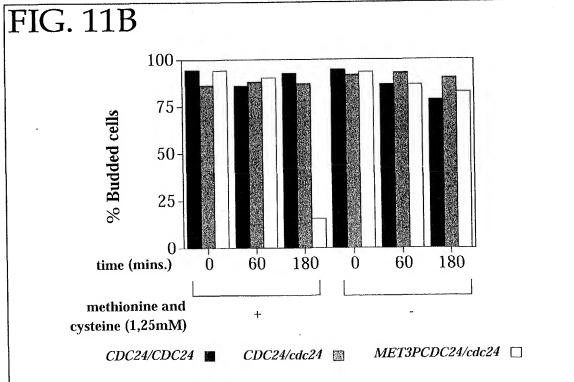


N FOTIDE SEQUENCES AND PROTEIN VENCES

Nern et al.

Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1





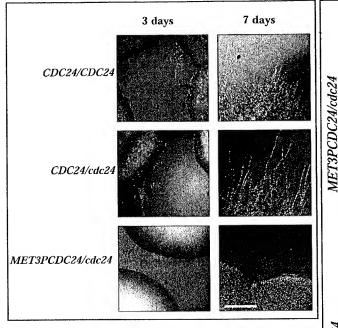


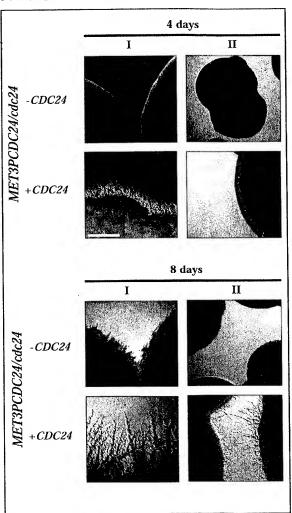
N' L'EOTIDE SEQUENCES AND PROTEIN VUENCES Nern et al. Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1

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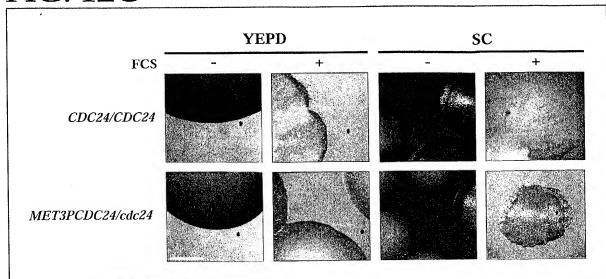
## FIG. 12A

## FIG. 12B





## FIG. 12C





N' EOTIDE SEQUENCES AND PROTEIN S VENCES Nem et al. Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1

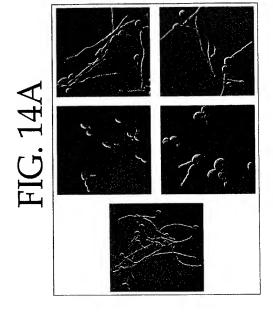


FIG. 14B

100

250

Wild-type cdc24 cdc42 budl bem1

Strain

FIG. 13A

wild-type

cate24

Cate24

Cate24

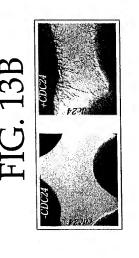
Cate24

Cate24

Cate24

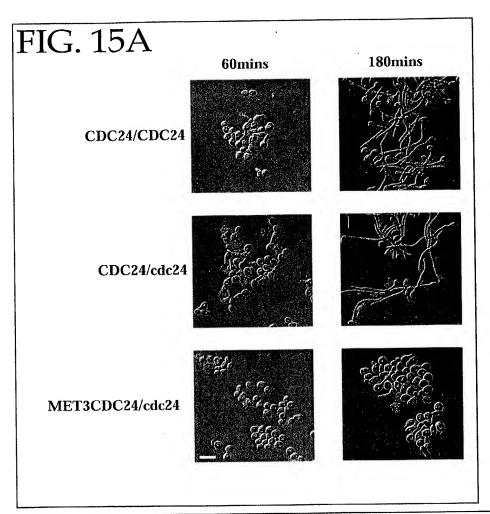
Cate27

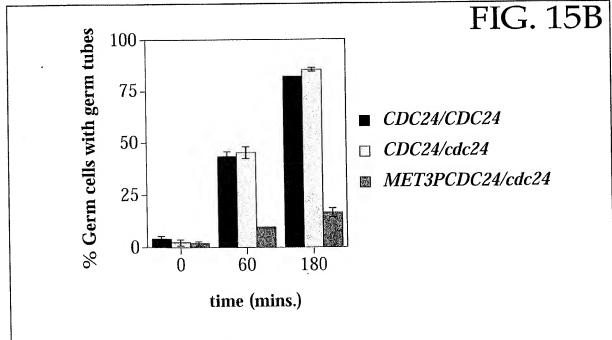
C





N' EOTIDE SEQUENCES AND PROTEIN UENCES Nem et al. Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1







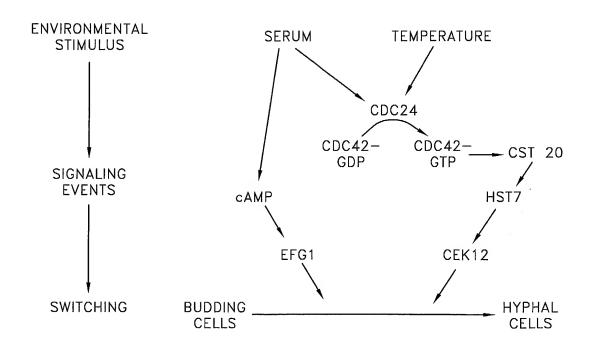


FIG. 16



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FIG. 17<sub>A</sub>

FIG. 17<sub>B</sub>

FIG. 17<sub>c</sub>

FIG. 17<sub>D</sub>

FIG. 17<sub>E</sub>

FIG. 17



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cdc24 (wt)

5

SEQ. I.D. NO:1

DNA:

tatattgggctgcaagaaacactttgcatttaacgatgaggagcttttcactatatccgacgtttttgccaactcgacgtcccagctggtcaaagt10 gctagaagtagtagaaacgctaatgaattccagc

SEQ. I.D. NO:2

Protein:

PLCILFNSVKPQFKLPVIASDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ 15 LVKVLEVVETLMNSS

В. cdc24-m1

20 SEQ. I.D. NO:3

DNA:

ccctctgtatacttttcaactctgtgaagccgcaatttaaattaccggtaatagcatttgacgatttgaaagtctgtaaaaaaatccatttatgacttta tattgggctgcaagaaacactttgcatttaacgatgaggagcttttcactatatccgacgtttttgccaactcgacgtcccagctggtcaaagtgctagaagtagtagaaacgctaatgaattccagc

25

SEQ. I.D. NO:4

Protein:

PLCILFNSVKPQFKLPVIAFDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ LVKVLEVVETLMNSS

30



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FIG.  $17_{\rm B}$ 

C. cdc24-m2

SEQ. I.D. NO:5

DNA:

ccctctgtatacttttcaactctgtgaagccgcaatttaaattaccggtaatagcatctggcgatttgaaagtctgtaaaaaatccatttatgactttatattgggctgcaagaaacactttgcatttaacgatgaggagcttttcactatatccgacgtttttgccaactcgacgtcccagctggtcaaagt gctagaagtagtagaaacgctaatgaattccagc

10

SEQ. I.D. NO:6

Protein:

PLCILFNSVKPQFKLPVIASGDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ LVKVLEVVETLMNSS

15

D. cdc24-m3

SEQ. I.D. NO:7

DNA:

cccctctgtatacttttcaactctgtgaagccgcaatttaaattaccggtaatagcacctgacgatttgaaagtctgtaaaaaatccatttatgactt20 gctagaagtagtagaaacgctaatgaattccagc

SEQ. I.D. NO:8

25 Protein:

> PLCILFNSVKPQFKLPVIAPDDLKVCKKSIYDFILGCKKHFAFNDEELFTISDVFANSTSQ LVKVLEVVETLMNSS



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FIG. 17<sub>C</sub>

SEO ID NO. 10

STE4 DNA sequence (wild-type)

ATGGCACATCAGATGGACTCGATAACGTATTCTAATAATGTCACCCAACAGTATATACAACCACAAAGTCTACAGGA TATCTCTGCAGTGGAGGAAGAATTCAAAATAAAATAGAGGCCGCCAGACAAGAGAGTAAACAGCTTCATGCTCAAATAA ATAAAGCAAAACACAAGATACAAGATGCAAGCTTATTCCAGATGGCCAACAAAGTTACTTCGTTGACCAAAAATAAGATC AACTTAAAGCCAAATATCGTGTTGAAAGGCCATAATAATAAAATCTCAGATTTTCGGTGGAGTCGAGATTCAAAACGTAT TTTGAGTGCAAGTCAAGATGGCTTTATGCTTATATGGGACAGTGCTTCAGGTTTAAAACAGAACGCTATTCCATTAGATT CTCAATGGGTTCTTTCCTGCGCTATTTCGCCATCGAGTACTTTGGTAGCAAGCGCAGGATTAAACAATAACTGTACCATT TATAGAGTTTCGAAAGAAAACAGAGTAGCGCAAAACGTTGCGTCAATTTTCAAAGGACATACTTGCTATATTTCTGACAT TGAATTTACAGATAACGCACATATATTGACAGCAAGTGGGGATATGACATGTGCCTTGTGGGATATACCGAAAGCAAAGA GGGTGAGAGAATATTCTGACCATTTAGGTGATGTTTTGGCATTAGCTATTCCTGAAGAGCCCAAACTTAGAAAATTCTTCG 10 CGTTAACGATAGTGATATTAATGCACTTCGTTTTTTCAAAGACGGGATGTCGATTGTTGCAGGAAGTGACAATGGTGCGA TAAATATGTATGATTTAAGGTCGGACTGTTCTATTGCTACTTTTTCTCTTTTTTCGAGGTTATGAAGAACGTACCCCTACC CCTACTTATATGGCAGCTAACATGGAGTACAATACCGCGCAATCGCCACAAACTTTAAAATCAACAAGCTCAAGCTATCT AGACAACCAAGGCGTTGTTTCTTTAGATTTTAGTGCATCTGGAAGATTGATGTACTCATGCTATACAGACATTGGTTGTG 15 TTGTGTGGGATGTATTAAAAGGAGAGATTGTTGGAAAATTAGAAGGTCATGGTGGCAGAGTCACTGGTGTGCGCTCGAGT CCAGATGGGTTAGCTGTATGTACAGGTTCATGGGACTCAACCATGAAAATATGGTCTCCAGGTTATCAATAG

SEQ ID NO. 11 20 Ste4 Protein sequence (wild-type)

MAHQMDSITYSNNVTQQYIQPQSLQDISAVEEEIQNKIEAARQESKQLHAQINKAKHKIQDASLFQMANKVTSLTKNKIN LKPNIVLKGHNNKISDFRWSRDSKRILSASQDGFMLIWDSASGLKQNAIPLDSQWVLSCAISPSSTLVASAGLNNNCTIY  ${\tt RVSKENRVAQNVASIFKGHTCYISDIEFTDNAHILTASGDMTCALWDIPKAKRVREYSDHLGDVLALAIPEEPNLENSSN}$ 25 TFASCGSDGYTYIWDSRSPSAVQSFYVNDSDINALRFFKDGMSIVAGSDNGAINMYDLRSDCSIATFSLFRGYEERTPTP TYMAANMEYNTAQSPQTLKSTSSSYLDNQGAVSLDFSASGRLMYSCYTDIGCVVWDVLKGEIVGKLEGHGGRVTGVRSSP DGLAVCTGSWDSTMKIWSPGYQ

30

SEQ ID NO. 12 ste4-o15 DNA sequence (mutant)

ATGGCACATCAGATGGACTCGATAACGTATTCTAATAATGTCACCCAACAGTATATACAACCACAAAGTCTACAGGA 35 TATCTCTGCAGTGGAGGAGAAATTCAAAATAAAATAGAGGCCGCCAGACAAGAGAGTAAACAGCTTCATGCTCAAATAA ATAAAGCAAAACACAAGATACAAGATGCAAGCTTATTCCAGATGGCCAACAAAGTTACTTCGTTGACCAAAAATAAGATC AACTTAAAGCCAAATATCGTGTTGAAAGGCCATAATAATAAAATCTCAGATTTTCGGTGGAGTCGAGATTCAAAACGTAT



10

20

30

35

# NEOTIDE SEQUENCES AND PROTEIN SECUNCES Nern et al. Appl. No.: 10/054,399 Atty Docket: DYOU13.1A2CP1

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FIG.  $17_D$ 

SEQ ID NO. 13

15 Ste4-o15 Protein sequence (mutant)

MAHQMDSITYSNNVTQQYIQPQSLQDISAVEEEIQNKIEAARQESKQLHAQINKAKHKIQDASLFQMANKVTSLTKNKIN
LKPNIVLKGHNNKISDFRWSRDSKRILSASQDGFMLIWDSASGLKQNAIPLDSQWVLSCAISPSSTLVASAGLNNNCTIY
RVSKENRVAQNVASIFKGHTCYISDIEFTDNAHILTASGDMTCALWDIPKAKRVREYSDHLGDVLALAIPEEPNLENSSN
TFASCGSDGYTYIWDSRSPSAVQSFYVNDSDINALRFFKDGMSIVAGSDNGAINMYDLRSDCSIATFSLFRGYEERTPTP
TYMAANMEYNTAQSPQTLKSTSSSYLDNQGAVSLDFSASGRLMYSCYTDIGCVVWDVLKGEIVGKLEGHGGRVTGVRSSP
DGLAVCTGSWDSTMKIWSPGYQ

SEO ID NO. 14

25 ste4-o17 DNA sequence (mutant)



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## FIG. 17<sub>E</sub>

TAAATATGTATGATTTAAGGTCGGACTGTTCTATTGCTACTTTTTCTCTTTTTCGAGGTTATGAAGAACGTACCCCTACC
CCTACTTATATGGCAGCTAACATGGAGTACAATACCGCGCAATCGCCACAAACTTTAAAATCAACAAGCTCAAGCTATCT
AGACAACCAAGGCGTTGTTTCTTTAGATTTTAGTGCATCTGGAAGATTGATGTACTCATGCTATACAGACATTGGTTGTG
TTGTGTGGGATGTATTAAAAGGAGAGATTGTTGGAAAAATTAGAAGGTCATGGTGGCAGGTCACTGGTGTGCGCTCGAGT
CCAGATGGGTTAGCTGTATGTACAGGTTCATGGGACTCAACCATGAAAATATGGTCTCCAGGTTATCAATAG

SEQ ID NO. 15

Ste4-o17 Protein sequence (mutant)

10 MAHQMDSITYSNNVTQQYIQPQSLQDISAVEEEIQNKIEAARQESKQLHAQINKAKHKIQDASLFQMANKVTSLTKNKIN
LKPNIVLKGHNNKISDFRWSRDSKRILSASQDGFMLIWDSASGLKQNAIPLDSQWVLSCAISPSSTLVASAGLNNNCTIY
RVSKENRVAQNVASIFKGHTCYISDIEFTDNAHILTASGDMTCALWDIPKAKRVREYSDHLGDVLALAIPEEPNLENSSN
TFASCGSDGYTYIWDSRSPSAVQSFYVNDSDINALRFFKDGMSIVAGSDNGAINMYDLRSDCSIATFSLFRGYEERTPTP
TYMAANMEYNTAQSPQTLKSTSSSYLDNQGAVSLDFSASGRLMYSCYTDIGCVVWDVLKGEIVGKLEGHGGRVTGVRSSP
DGLAVCTGSWDSTMKIWSPGYQ

SEQ I.D. No:16 refers to the epitope sequence "Tyr Pro Tyr Asp Val Pro Asp Tyr Ala".

SEQ I.D. No:17 refers to TEV protease recognition sequence "Gln Asn Leu Tyr Phe Gln Gly".

SEQ I.D. No:18 refers to peptide sequence "QFKLPVIAFDDLKVCKKSI".

SEQ I.D. No:19 refers to peptide sequence "QFKLPVIASGDLKVCKKSI".

SEQ I.D. No: 20 refers to peptide sequence "QFKLPVIAPDDLKVCKKSI".

SEQ I.D. No:21 refers to peptide sequence "QFKLPVIASDDLKVCKKSI".

SEQ I.D. No:22 refers to peptide sequence "QYEFDVILSPELKVQMKTI".